I should like to make plain my gratitude to Dr. Joseph R. Bove. Asked to become advisor, he responded by becoming advisor, teacher and friend.

J.M.D.

ISOIMMUNIZATION BY TRANSFUSION

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"The principal facts concerning this subject are that normal sera may agglutinate or hemolyze the erythrocytes of other individuals of the same species, and that on injection of red cells antibodies may be formed which by agglutination or hemolysis differentiate the blood corpuscles of various individuals within a species."

---Karl Landsteiner.

INTRODUCTION

This paper is in two parts. Part I is a review of the activities and experience of the Blood Transfusion Service of Yale-New Haven Hospital. Since 1961 the service has kept accurate records of its experience in antibody detection and identification by serological methods. With a record of five full years available it seemed appropriate to make a careful analytical review. It was hoped that such a review might reveal something more about the frequency of antibody occurrence, might make possible some predictions based on the experience of the service, and might make it possible to compare the experience of this service with that of other blood transfusion services.

With these general aims the review was undertaken. During the course of the analysis it became clear that while the records showed how many antibodies the service had detected and identified they did not show how many antibodies had been caused by transfusions administered in this hospital. There had never been a systematic follow-up of a random group of transfused patients to determine how many of them developed irregular blood group antibodies in response to the antigenic stimulus of therapeutic transfusion. A review of the literature indicated that no other transfusion service had done such a study either. Asked as simply as possible, the question was, "What are the chances that a patient who is transfused in this hospital will

develop an irregular blood group antibody as a result of the transfusion?" Part II of this paper describes the experiment that was designed and carried out in the hope of finding an answer to this question.

I. THE BLOOD TRANSFUSION SERVICE, 1961-1965

Donors and Patients

Donors

Bank blood used in Connecticut is given to hospitals by the Connecticut Red Cross. The Red Cross collects the blood from volunteer donors who are resident in the state and distributes it through its central facility in Hartford. The volunteer donors meet the standards of the American Association of Blood Banks (21,28) but are otherwise unselected as to age, sex or race. There are no commercial blood banks in this state. The assumption is that a donor who cannot profit from his donation will be less likely to misrepresent the state of his health than will a person who is selling his blood for cash. The implication for the patient population is that there will be a low incidence of serum hepatitis associated with transfusion.

In general, the donor bloods used in this hospital are supplied by the Connecticut Red Cross. However, a certain number of donor bloods are drawn in this hospital, either for open heart surgery or to meet emergency needs. Except for a few very rare blood types these bloods too are given by volunteers

who are unselected except that they must meet the standards of the American Association of Blood Banks.

Nonobstetrical Hospital Patients

These are adult and pediatric medical and surgical patients whose blood is typed and tested for antibodies in the expectation that they will be transfused. Typing and antibody detection testing is not routinely performed on the blood of every patient admitted to this hospital.

Obstetrical Patients

The blood of every pregnant woman is typed and tested for the presence of irregular blood group antibodies when she is first seen in the obstetrical clinic of this hospital.

Patients in Other Hospitals

When asked to do so, this service tries to assist other hospitals in this region with occasional difficult crossmatches or with identification of obscure antibodies.

Antibody Detection Test

All donor bloods, whether received from the Connecticut Red Cross or drawn in this hospital, and the bloods of all patients who are considered for transfusion therapy are subjected to an antibody detection test. Throughout most of the period under consideration the antibody detection method was constant. It consisted of tests performed in a saline medium and in an albumin-enriched saline medium to which antihuman globulin was added:

Saline method. One drop of the serum to be screened is put into a 10x75 mm test tube and one drop of a 4% suspension in saline of this service's Reagent Red Blood Cells (21,28) described in Part II is added. The tube is centrifuged for 10 seconds at 3,400 rpm in a MacBick Hemofuge® and examined macroscopically for agglutination. The tube is then allowed to stand at room temperature for 15 minutes. It is then again centrifuged for 10 seconds at 3,400 rpm and examined macroscopically for agglutination.

Albumin-antiglobulin method. One drop of the serum to be screened is put into a 10x75 mm test tube. One drop of a 4% suspension in saline of Reagent Red Blood Cells and two drops of a 22% solution of bovine albumin are added to the tube. The tube is then centrifuged for 10 seconds and examined macroscopically for agglutination. The tube is then placed in a 37° C. water bath for 15 minutes, centrifuged for 10 seconds and examined macroscopically. The cells are then washed three times with saline. After the last wash a drop of antihuman globulin (Coombs serum) is added to the cells and the suspension is thoroughly mixed. The tube is then centrifuged for 10 seconds and examined macroscopically and microscopically.

In mid-1965 the saline method was eliminated from the antibody detection procedure and a proteolytic enzyme method was instituted:

Enzyme method. One drop of the serum to be screened is put into a 10x75 mm test tube and one drop of a 4% suspension in saline of Reagent Red Blood Cells is added. Next a drop of commercial proteolytic enzyme preparation (Spectrazyme) is added, and the tube is allowed to stand three minutes at room temperature. It is then centrifuged for 10 seconds and examined macroscopically for agglutination. The tube is then placed in the 37° C. water bath and allowed to remain there until the albumin-antiglobulin tube procedure has been completed (about 20 minutes). It is then centrifuged for 10 seconds and examined macroscopically.

Results

Transfusions and Recipients

During the five years covered by this review, 54,771 units of blood and blood products were administered to 14,783 patients for an average of 3.70 units per transfused patient over the entire five-year period. The figures for each year are shown in Table 1, and the same data are shown graphically in Figure 1.

Table 2 shows the per cent of each year's admissions that received transfusions and compares the annual figures with the mean per cent for the five-year period. As presented, the differences in percentages are highly significant. It must be pointed out, however, that the table compares the number of patients transfused in calendar years with the number of

patients admitted in fiscal years. This is not completely accurate, but it is felt to be a justifiable approximation upon which to base general conclusions.

There is an overall upward trend in the number of transfusions, the number of recipients and the percentage of patients transfused from 1961 to 1965. The number of units administered was 9,971 in 1961 and was 11,785 in 1965, an increase of 18% from the beginning to the end of the period under study. The number of recipients was 2,662 in 1961 and 3,215 in 1965, an increase of 21% from the beginning to the end of the period under study. In fiscal 1961 there were 28,439 admissions and in fiscal 1965 there were 30,244 admissions, an increase of 6.4% This upward trend, however, is not an unbroken one -- there were fewer recipients in 1964 than in 1963, there were fewer transfusions given in 1963 than in 1962, and a smaller percentage of patients was transfused in 1964 than in 1963.

Deliveries

Pregnancy is the other great source of sensitization to blood group factors with the resultant production of circulating irregular blood group antibodies. An insignificant number of the women who deliver in this hospital have ever been transfused, so that for analytical purposes the occurrence of antibodies in obstetrical patients can be compared with the total number of deliveries. The number of deliveries in the years under study is shown in Table 3. A total of 24,876 babies was delivered in the five-year period.

TRANSFUSIONS and RECIPIENTS

	1961	1962	1963	1964	1965	Total
Transfusions	9,971	10,886	10,860	11,269	11,785	54,771
Recipients	2,662	3,000	3,131	2,775	3,215	14,783
$\frac{\text{Total no. of units given}}{\text{Total no. of recipients}}$	3.74	3.63	3.47	4.06	3.66	3.70

Table 1

TRANSFUSIONS and RECIPIENTS

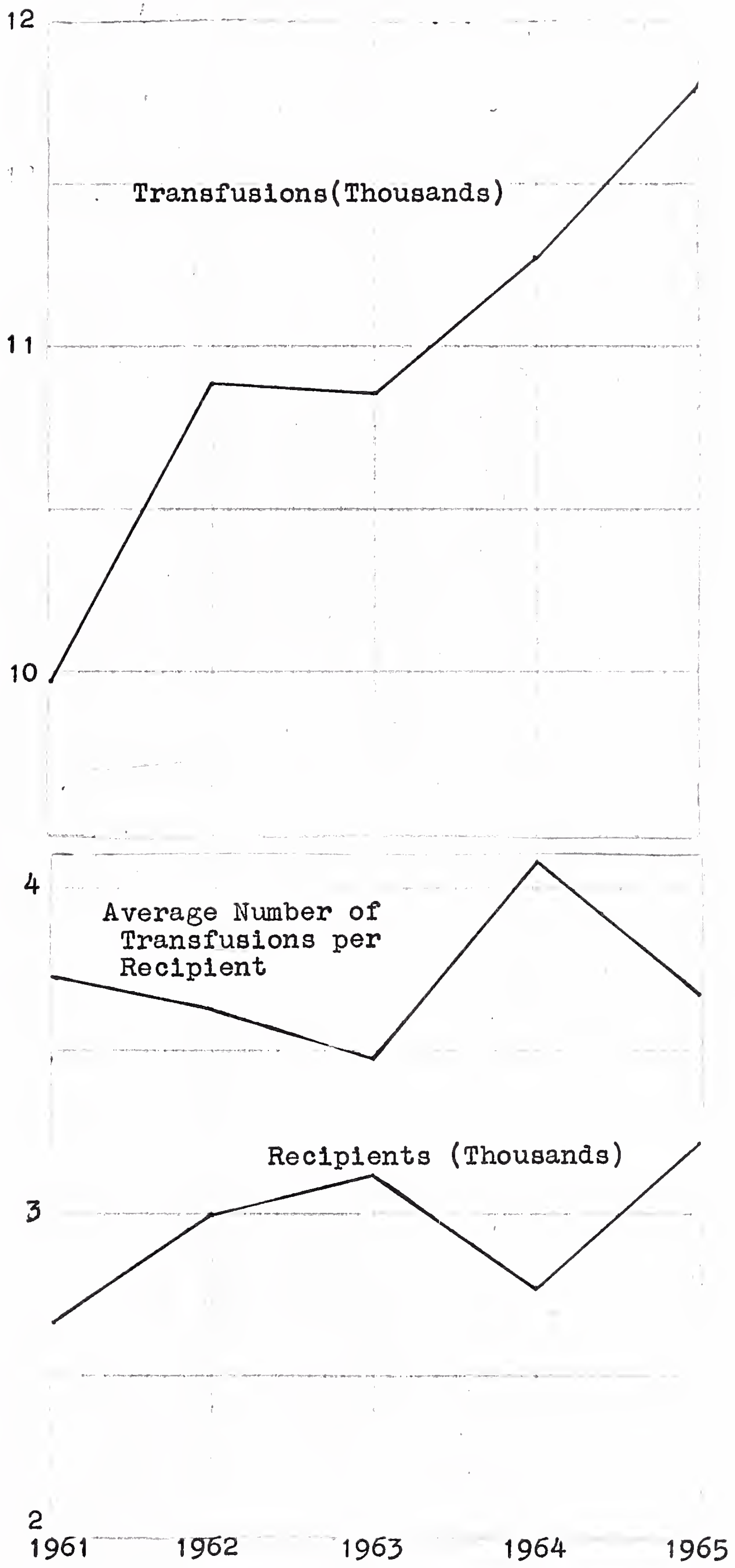

FIGURE 1

PERCENT OF PATIENTS TRANSFUSED

	1961	1962	1963	1964	1965	Total
Patients transfused	2,662	3,000	3,131	2,775	3,215	14,783
Patients not transfused	25,777	25,548	26,000	26,780	27,029	131,134
Patients admitted*	28,439	28,548	29,131	29,555	30,244	145,917
% transfused	9.36	10.51	10.75	9.39	10.63	10.13

$X^2_{(3)} = 61.2294 \qquad P \ll 0.001$

TABLE 2

Statistical analysis by Dr. Paul S. Anderson, Jr., Department of Epidemiology and Public Health, Yale University School of Medicine.

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* Admission figures are for the hospital's fiscal year, which ends September 30. All other data in this paper are for the calendar years.

DELIVERIES

	1961	1962	1963	1964	1965	Total
Deliveries	5,305	4,915	5,013	4,872	4,771	24,876

TABLE 3

Numbers of Antibodies Identified

During the period under review the Blood Transfusion Service identified a total of 1,057 antibodies.* Table 4 shows the distribution of these antibodies by years and according to the categories of the persons in whose serums they were found. Table 5 is a complete list of the antibodies, arranged according to specificity and frequency of occurrence. Over the entire five-year period 25% of the identified antibodies were found in donor bloods, 38% were found in the serums of nonobstetrical patients in this hospital, 32% were found in the serums of obstetrical patients in this hospital and 5% were found in serums studied for other hospitals.

Table 6 shows the rate of identification of antibodies in the various categories -- donor bloods, nonobstetrical transfused patients in this hospital and obstetrical patients in this hospital. In each category the overall trend is upward; that is, there is a general increase in the rate of antibody identification between 1961 and 1965. In 1965, however, the rate of antibody identification dropped slightly from the 1964 level in all categories. Figure 2 shows these data graphically.

Frequency of Various Antibodies

Table 7 shows, for each of the years under consideration, the frequency of each of the six most commonly identified antibodies -- D, Le^a, K, C+D, P, and E. Taken together, these antibodies constitute 83% of all the antibodies identified in

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* This figure represents only identified antibodies; it does not include nonspecific cold agglutinins and it does not include nonsensical results with panels of red cells.

NUMBERS OF ANTIBODIES IDENTIFIED

	1961	1962	1963	1964	1965	Total	%
Donor Bloods	14	56	49	74	74	267	25
Nonobstetrical Hospital Patients	18	33	95	130	120	396	38
Obstetrical Patients	33	73	36	105	95	342	32
Other Hospitals	9	8	13	8	14	52	5
TOTAL	74	170	193	317	303	1,057	100

TABLE 4

ANTIBODIES IDENTIFIED
1961 - 1965

ANTIBODY	1961	1962	1963	1964	1965	Total
D	44	89	91	107	91	422
Le^a	3	20	23	55	34	135
K	5	17	22	33	22	99
C+D	3	12	14	20	33	82
P	2	2	1	38	29	72
E	5	7	16	15	20	63
Fy^a	2	7	7	6	9	31
c	0	8	3	6	5	22
Le^b	0	0	2	7	8	17
M	2	2	3	5	4	16
Le^a+Le^b	0	1	0	4	9	14
C	0	0	2	4	3	9
e	2	0	2	1	1	6
E+K	0	0	1	2	3	6
c+E	0	0	0	1	4	5
C+D+E	0	1	0	1	3	5
D+E	0	1	2	1	0	4
Jk^a	0	1	2	0	0	3
D+K	1	0	0	1	1	3
Jk^b	0	1	0	0	1	2
C^w	1	0	0	1	0	2
C+D+Fy^a	0	1	0	0	1	2
M+K	0	0	0	0	2	2
C+e	0	0	0	0	2	2
S	0	0	0	1	1	2
N	0	0	0	1	1	2
C^w+E	0	0	0	0	2	2
c+E+Fy^a	1	0	0	0	0	1
c+K	0	0	0	0	1	1
c+E+K+Fy^a	0	0	0	0	1	1
c+E+Fy^b	0	0	0	1	0	1
c+e	0	0	0	1	0	1
c+Jk^a	0	0	0	0	1	1
C+E	1	0	0	0	0	1
C+K	0	0	0	1	0	1
C+D+K	0	0	0	0	1	1
D+Le^b	0	0	0	0	1	1
D+Fy^a	0	0	0	0	1	1
D+P	0	0	0	0	1	1
Bi^a	1	0	0	0	0	1
M+P	0	0	0	1	0	1
E+Fy^a	0	0	0	1	0	1
Le^a+Lu^a	0	0	0	0	1	1

TABLE 5

(cont'd)

Table 5 (cont'd)

ANTIBODY	1961	1962	1963	1964	1965	Total
$K+K^w$	0	0	0	0	1	1
$L+Lu^a$	0	0	0	0	1	1
O(H)+P	0	0	0	0	1	1
Du^a	0	0	1	0	0	1
K^w	0	0	1	0	0	1
Jk^a+P	0	0	0	1	0	1
$K+Le^a$	0	0	0	1	0	1
Wr^a	0	0	0	0	1	1
Lu^b	0	0	0	0	1	1
$K+Fy^a+Lu^a$	0	0	0	0	1	1
hr''^S	1	0	0	0	0	1
	74	170	193	317	303	1057

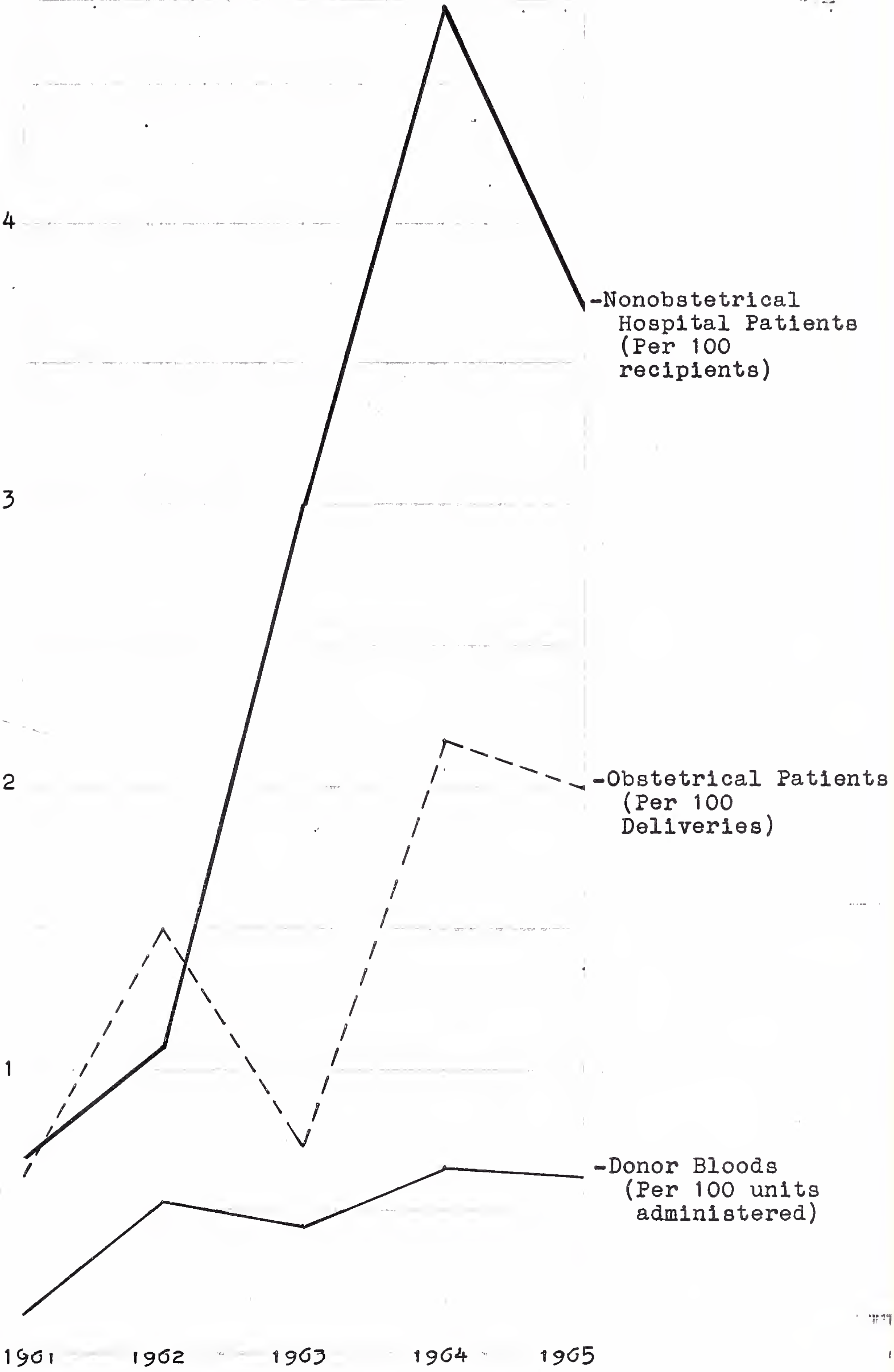
TABLE 5

(Concluded)

RATE OF ANTIBODY IDENTIFICATION

	1961	1962	1963	1964	1965	Five-year average
Donor bloods $\left(\frac{\text{units with antibody}}{\text{100 units administered}}\right)$	0.14	0.51	0.45	0.65	0.63	0.49
Obstetrical Patients $\left(\frac{\text{Patients with antibody}}{\text{100 deliveries}}\right)$	0.62	1.5	0.72	2.16	2.0	1.35
Nonobstetrical Hospital Patients $\left(\frac{\text{Patients with antibody}}{\text{100 recipients}}\right)$	0.68	1.1	3.0	4.78	3.73	2.7

TABLE 6



RATE of ANTIBODY IDENTIFICATION

FIGURE 2

FREQUENCY OF VARIOUS ANTIBODIES

	1961		1962		1963		1964		1965		Totals	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
D	44	59	89	52	91	47	107	34	91	30	422	40
Le^a	3	4	20	12	23	12	55	17	34	11	135	13
K	5	7	17	10	22	11	33	10	22	7	99	9
C+D	3	4	12	7	14	7	20	6	33	11	82	8
P	2	3	2	1	1	0.5	38	12	29	10	72	7
E	5	7	7	4	16	8	15	5	20	7	63	6
TOTALS	62	84	147	86	167	87	268	85	229	76	873	83

TABLE 7

the period under review. Anti-D, expectedly, is by far the most frequently occurring antibody. But it ranges from a high of 59% of all antibodies identified by the service in 1961 to a low of 30% of all antibodies identified by the service in 1965. This percentage decline is offset by absolute or percentage increases in others of the most commonly identified antibodies, particularly anti-Le^a, anti-P and anti-C+D. Figure 3 shows graphically the percentage of each year's total antibody yield that was represented by each of the six commonest antibodies. Anti-D, of course, towers over the rest. But comparison of the percentage of anti-D in each year's antibody total with the combined percentages of the next five reveals a clear reciprocal relationship (Figure 4).

Comparison With What Others Have Found.

Little data has been published that will allow comparison of the experience of this service with that of other transfusion services. Reports of antibody occurrence have been published (5,8,12,13,14,15,17,18,19,20), but virtually all of them deal with selected populations. Myhre et al (27) have reported on the incidence of irregular antibodies in the serums of blood donors, and the experience of this service may be compared with the experience of Myhre's group, at least as far as donor bloods are concerned. Table 8 shows the frequency in donor bloods of the six commonest antibodies identified by this service in each of the years under review. Taken together, these six antibodies constitute 83% of the antibodies identified by this service in donor bloods during the period under review and 88% of the antibodies identified in donor

THE SIX COMMONEST ANTIBODIES

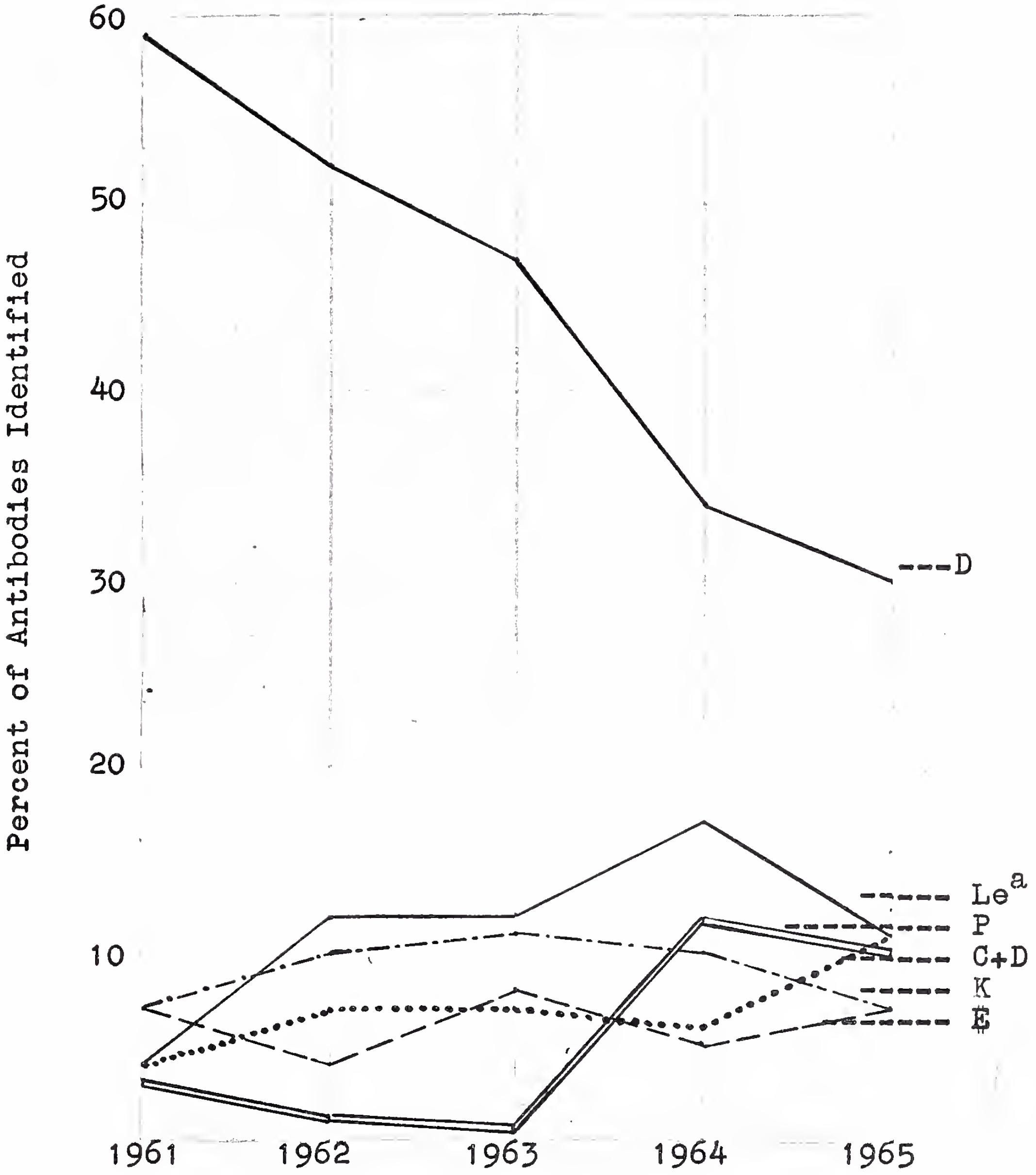
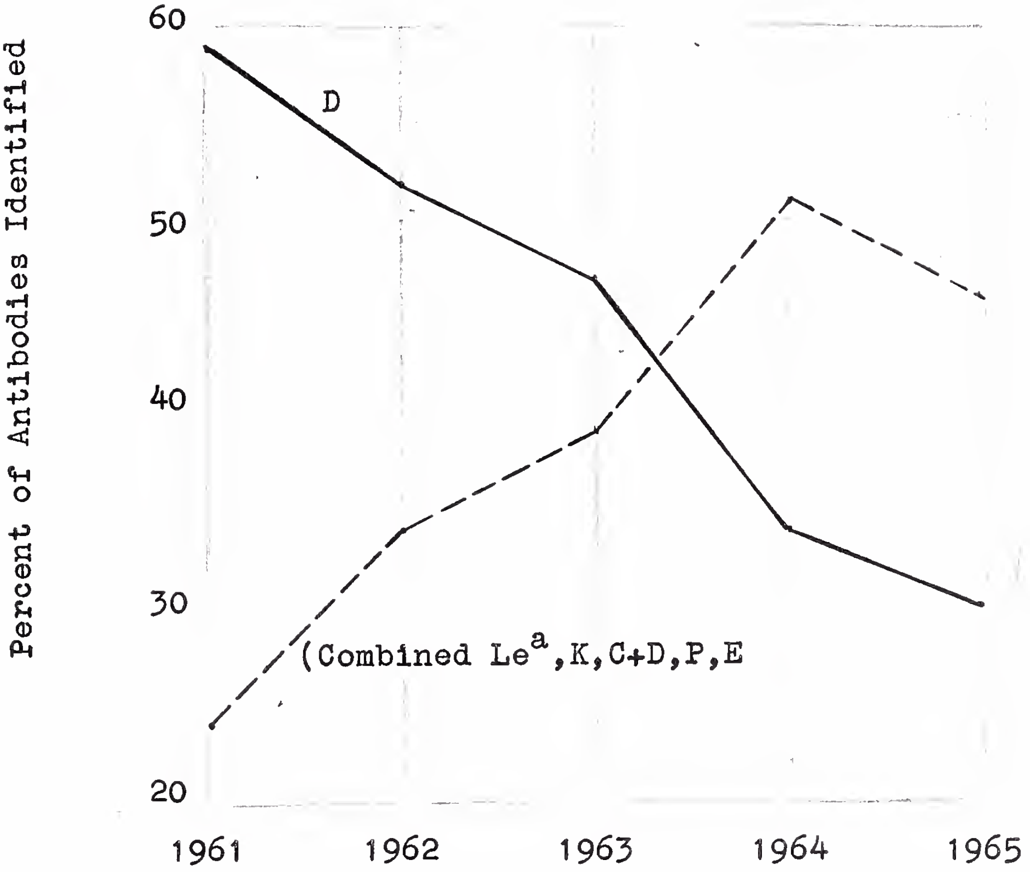


FIGURE 3

ANTI-D AND THE NEXT FIVE COMMONEST ANTIBODIES



ANTIBODY FREQUENCY
in
DONOR BLOODS ONLY

	1961	1962	1963	1964	1965	Total	%*	Myhre* %
D	8	33	24	17	26	108	40	37
Le^a	1	6	3	10	7	27	10	4
K	1	4	4	8	4	21	8	12
C+D	0	4	5	7	8	24	9	25
P	1	0	1	18	13	33	12	1
E	0	3	2	3	1	9	3	10
Totals	11 (79%)	50 (89%)	39 (80%)	63 (85%)	59 (80%)	222	83	88

TABLE 8

* The Myhre group (27) found that 0.34% of its donor bloods contained isoimmune antibodies; the Yale-New Haven service found that 0.49% of its donor bloods contained isoimmune antibodies; as these overall figures are similar, it is felt that the percentages in the last two columns of this table are legitimately comparable.

bloods in the Myhre et al study. For comparison purposes the last two columns of the table are the interesting ones.

Childers et al (31) tested 19,296 blood donors at Southwest Blood Banks, Inc., Scottsdale, Arizona. They found identifiable irregular isohemagglutinins in 2.76% of this group, but only 0.62% had titers of 32 or greater.

Smith et al (32) studied 12,297 obstetrical patients for irregular blood group antibodies. They found 173 anti-D and 85 specific irregular antibodies, for a total of 258 specific antibodies. This calculates out to an occurrence rate of 2.1 antibodies per hundred patients studied.

Conclusions

Transfusions and Recipients

During the entire five-year period an average of 3.70 units was given to each patient who was transfused. The annual figures vary only slightly from the five-year average, so that in general the number of units given to each patient has stayed constant throughout the period of review.

From 1961 to 1965, while the total number of units administered increased by 18% and the total number of recipients increased by 21%, the number of hospital admissions increased by only about 6%. Thus it would appear at first glance that the therapeutic use of blood in this hospital is increasing about three times as fast as the number of admissions to the hospital. In fact the situation is not that simple. Table

2 compares the per cent of each year's admissions that was transfused with the mean for the five-year period and shows that the differences are highly significant. But because the per cent of patients transfused did not increase each year, it is impossible to conclude with certainty that the use of transfusion therapy is increasing more rapidly than the number of admissions. This conclusion is suggested, however, and the question will be settled by the data of the next few years.

Numbers of Antibodies Identified

On the average, 13% of the donor bloods collected by the Connecticut Red Cross are from repeat donors; that is, from persons who have donated blood at some other time in the past. Blood from donors who are known to have circulating irregular blood group antibodies is not shipped to the hospitals. Nonetheless, 25% of all antibodies identified by this service were found in donor bloods. It is clear from this figure that a blood transfusion service must screen donor bloods as carefully as it tests the blood of patients who are to receive transfusions. This careful testing of donor bloods for antibody has two aims -- first, it prevents transfusion reactions that might occur as the result of action of donor antibody on recipient red cells; second, it is a method of identifying and removing from the donor pool those persons whose blood contains circulating antibody. Furthermore, services that perform careful antibody detection tests on donor

bloods are, in the opinion of most authorities, able to eliminate the minor crossmatch from their compatibility testing procedures (21,29).

The figures for the rates of antibody identification in the various groups are shown in Table 6. The materials and methods now in use by this service were instituted in 1961. The general increase in the rate of antibody identification from 1961 to 1964 probably reflects the increasing technical skill of the service during this time rather than any change in the nature of the patients and donors. It seems likely that the figures for 1964 and 1965 are more suggestive of what the service can expect in the future than are the figures from the earlier years. Thus it seems to fair to predict that in the next few years:

1) about 0.6% of the donor bloods received by this service will contain isoimmune antibodies;

2) about 2% of the women who deliver children in this hospital will have circulating isoimmune antibodies;

3) about 4% of the nonobstetrical hospital patients who receive transfusions in this hospital will have circulating isoimmune antibodies either before or after transfusion.

Frequency of Various Antibodies

Figure 4 shows graphically that anti-D represents a shrinking proportion of all antibodies identified by the service as other less common antibodies represent a growing

percentage. This was particularly true in the years 1961-1963 and as in the matter of the rate of antibody identification probably reflects the increasing technical skill of the service. In this case the increasing skill shows itself as increasingly frequent identification of less common antibodies. In this regard, two specific points deserve special comments. First, the frequency of anti-Le^a declined from 17% in 1964 to 11% in 1965 (Table 7). It was in mid-1965 that the room temperature saline method was discontinued by the service and the proteolytic enzyme method was begun. Anti-Le^a is maximally reactive in a saline medium at room temperature; it seems reasonable that the decreased frequency reflects the elimination from the antibody detection test of the medium in which this antibody is most highly reactive. Second, it is worthy of note that the decreasing frequency of anti-D is partially offset by an increasing frequency of anti-C+D. Anti-D is a more highly reactive than anti-C. It is probable that until the service had achieved a considerable skill the active anti-D was found but the anti-C activity was overlooked.

Figures for 1964 and 1965 are quite similar and probably indicate what the service's experience will be in the future. Thus it is probable that in the next few years:

1) the six commonest antibodies -- D, Le^a, K, C+D, P, and E -- will constitute about 80% of all antibodies identified by the service;

2) anti-D will constitute about 32% of all antibodies identified;

3) the next five commonest antibodies will make up about 50% of all antibodies identified.

Comparison With What Others Have Found

Both for Myhre's group in its four-year study and for this service in its five-year review anti-D makes up about 40% of all antibodies identified in donor bloods. In other instances the experience of the two groups varies widely. In particular, this service finds a larger percentage of anti-Le^a and anti-P among its donors than the Myhre et al group found in its donors. Ten percent of the antibodies identified by this service in donor bloods were anti-Le^a, compared with 4% for the Myhre group. And 12% of the antibodies found by this service in donor bloods were anti-P, compared with 1% for the Myhre group. The reason for this difference is not clear. It may reflect some technical difference that is not apparent, for both these antibodies should be active in the screening methods the Myhre group describes.

The Myhre group found that 0.34% of its donor bloods contained isoimmune antibodies. This service found isoimmune antibodies in 0.49% of its donor bloods. These figures are comparable. Childers et al of the Scottsdale group have reported an occurrence rate of identifiable irregular antibodies that is about four times the rate achieved by this service in its best years.

The Smith group's rate of antibody occurrence in obstetrical patients is very close to the experience of this service during the last two years of the period under survey. This tends both to confirm the findings of this service and to reaffirm the inference drawn elsewhere that the service's increasing rate of antibody identification in the years 1961 through 1963 represented in part its increasing skill in antibody identification.

Summary

During the five years under review, about 12% of the nonobstetrical patients in this hospital received blood or blood products. Each patient who was transfused received an average of 3.70 units. From the first year of the five-year review period to the last the amount of blood and blood products given to patients increased by 18% and the number of patients receiving transfusions increased by 21% while the number of admissions increased by 6.4%. It is probable but not yet statistically certain that an increasing proportion of patients is receiving transfusion therapy.

During this period it was found that 0.49% of donor bloods contained antibodies, the bloods of 1.35% of obstetrical patients contained antibodies and the bloods of 2.7% of nonobstetrical transfused hospital patients contained antibodies. The six most common antibodies constituted 83% of all antibodies identified.

Certain predictions are made on the basis of the five-year experience of this service and certain comparisons are made with

the findings of Myhre et al in the Milwaukee Blood Center, and with the findings of others.

II. THE OCCURRENCE OF IRREGULAR BLOOD GROUP ANTIBODIES AFTER TRANSFUSION

Discovery of the ABO system and its naturally occurring antibodies by Landsteiner (1,2) in 1900 made widespread use of transfusion therapy possible. The discovery by Ehrlich and Morgenroth (3), made shortly thereafter, that erythrocytes from a goat when injected into a second goat will stimulate the second to make a hemolysin laid the basis for the discovery of the other blood group systems and for all current work in isoimmunization.

After Landsteiner's discovery of the ABO system more than twenty-five years elapsed before another system was discovered. Landsteiner and Levine (22) injected human red cells into rabbits and with the antibodies thus produced they identified the MN system in 1928. Landsteiner and Wiener (23,24) injected rhesus monkey red cells into rabbits and obtained antibodies that reacted with both human and monkey cells. Using these serums they identified the Rh system in 1940. Since 1940 eight other systems have been discovered, so that now one must contend with 11 major systems containing more than 100 known blood factors. Any one of these factors may stimulate antibody formation. And every antibody is a potential hazard to the patient in whose blood it circulates. To a woman in the child-bearing years an antibody means the danger of hemolytic disease in her children. To any patient who needs transfusion, a

circulating antibody means increased difficulty in finding compatible blood. And there is the subtler problem of the patient who is sensitized to one of the blood factors but in whom the antibody is not detectable serologically, either because it never reached detectable levels or because it has declined to a level at which it is no longer detectable. In either case this patient is peculiarly liable to suffer a delayed hemolytic transfusion reaction.

For these reasons it has become important to know something about the frequency of isoimmunization after transfusion. A number of investigators have worked on this problem and have brought a number of different methods to bear on it. Most of the studies (4,5,6,7,8,9,10) have dealt with selected populations. Some (11,12,13,14) have been based upon deliberate attempts to sensitize volunteers by injecting into them small quantities of incompatible red cells. Some studies have tested for the frequency of sensitization as a result of transfusion with specific red blood cell factors -- e.g., Rh (15,16) or Kell (17). Others have been limited to open heart surgery patients, who receive massive transfusions (18, 19,20).

The purpose of this study was to follow a random group of patients who were transfused under clinical conditions because they needed transfusions, and to find out how many of them made new irregular blood group antibodies as a result of these transfusions.

Patients

As far as practicable this study included all patients on the University service, medical and surgical, who received transfusions during a one-month period beginning July 14, 1964, and ending August 13, 1964, except:

1) those under 15 years of age and

2) those discharged before the fifth day after transfusion.

There were 111 patients brought into the study. They received a total of 376 units of blood. Individuals received from one to 22 units, for an average of 2.96 units per patient. All blood given these patients was ABO-compatible, Rh_o(D)-compatible and was found to be free of antibody when tested by the Blood Transfusion Service's usual methods (these methods are described in Part I of this paper).

In addition to the Blood Transfusion Service's routine pretransfusion testing, a pretransfusion sample of the patient's serum was tested for antibodies by the method of this study, which is described below. Blood samples were then drawn from each of these patients each five days after transfusion as long as they stayed in the hospital and an effort was made to secure a follow-up sample from each patient about a year after transfusion; that is, at some time during the months of July or August 1965. All these samples were tested for antibody by the methods of this study.

Methods

Antibody Detection

The antibody detection method used in this study consists of testing the ability of the patients' serums to agglutinate the Blood Transfusion Service's Reagent Red Blood Cells (21, 28) under various conditions: 1) in a saline medium at room temperature, 2) in a saline medium at 4° C., 3) in a saline medium at 37° C. to which proteolytic enzyme is added, 4) in an albumin-enriched saline medium after 30 minutes' incubation at 37° C., and 5) by the indirect antiglobulin method.

Seven members of the house and laboratory staffs make up the donor pool for the service's Reagent Red Blood Cells. Each batch of Reagent Red Blood Cells contains red cells from three of these seven donors, so that each required antigenic determinant is present on more than thirty per cent of the cells in the test suspension. Blood is drawn from the donors into modified Alsever's solution (30). The blood from all three donors is then mixed, and the mixture is stored at 4° C. For daily use in the blood bank, small aliquots are withdrawn and are washed three times in normal saline. The antigenic determinants on the red cells of the various members of this donor pool are shown in Table 9.

The antibody detection test is conducted with a saline tube, an enzyme tube and an albumin-antiglobulin tube:

Saline tube. One drop of the serum to be screened is put into a 10x75 mm test tube and one drop of a 4% suspension in

DONOR POOL
For the
REAGENT RED BLOOD CELLS

ANTIGEN	DONORS 1	2	3	4	5	6	7	POSSIBLE COMBINATIONS	REQUIRED ANTIGENS LACKING
ABO	0	0	0	0	0	0	0	1-2-3	None
Rh$_o$(D)	+	+	+	+	+	+	+		
rh'(C)	0	+	+	+	0	+	0	1-2-4	None
rh"(E)	+	+	0	+	+	+	+		
hr'(c)	+	+	+	+	+	+	+	1-2-6	None
hr"(e)	+	+	+	+	+	+	0		
K (Kell)	+	0	0	+	+	+	+	2-3-4	None
k (Cellano)	+	+	+	0	+	+	+		
Fy^a (Duffy)	+	+	+	+	+	0	0	2-3-6	None
Fy^b (Duffy)	0	+	+	0	+	+	+		
M	0	+	+	+	+	0	0	2-3-7	None
N	+	+	+	+	0	+	+		
S	0	+	0	0	+	0	0	2-4-5	None
s	+	+	+	+	+	+	+		
Le^a (Lewis)	0	0	+	+	0	+	0	2-4-6	None
Le^b (Lewis)	+	+	0	0	0	0	+		
Jk^a (Kidd)	0	+	+	+	+	+	+	2-4-7	None
Jk^b (Kidd)	+		0	+	0	+	+		
Lu^a (Lutheran)	0		0	0	0	0	0	2-5-6	None
Lu^b (Lutheran)	+		+	+	+	+	+		
P	+	0	+	+	+	+	+	2-6-7	None
Js^b(Matthews)	+	+	+	+	+	+	+		
Xg^a	0	+	0	+			0	4-5-7	None

TABLE 9

saline of the service's Reagent Red Blood Cells is added. The tube is allowed to stand for 20 minutes at room temperature. It is then centrifuged for 10 seconds at 3,400 rpm in a MacBick Hemofuge® and examined macroscopically for agglutination. The cells are then resuspended and the tube is refrigerated at 4° C. for 20 minutes. Then it is again centrifuged for 10 seconds and examined macroscopically.

Enzyme tube, method #1. This method was used with all samples acquired while the patients were hospitalized. One drop of the serum to be tested, one drop of a 4% suspension in saline of Reagent Red Blood Cells and one drop of papain (prepared by Low's method) are put into a 10x75 mm test tube. The tube is placed in a 37° C. water bath for 30 minutes, then centrifuged for 10 seconds at 3,400 rpm and examined macroscopically.

Enzyme tube, method #2. This method was instituted in 1965 and was used on all one-year follow-up serum samples. One drop of serum, one drop of 4% suspension of Reagent Red Blood Cells and one drop of a commercial enzyme solution (Spectrazyme) are put into a 10x75 mm test tube. The tube is allowed to stand at room temperature for 3 minutes. It is then centrifuged and examined macroscopically. If there is no agglutination in the tube the cells are completely resuspended and the tube is placed in a 37° C. water bath for 10 minutes. It is then again centrifuged and examined macroscopically.

Albumin-antiglobulin tube. One drop of serum, one drop of 4% suspension of Reagent Red Blood Cells and one drop of a 22% solution of bovine albumin are placed into a 10x75 mm test tube and mixed thoroughly. The tube is then centrifuged and examined macroscopically. The cells are then resuspended and the tube is placed in a 37° C. water bath for 30 minutes, then again centrifuged and examined macroscopically. The cells are again resuspended and are washed three times with normal saline. A drop of antiglobulin serum (University of Pennsylvania) is added to the cells and the suspension is thoroughly mixed. The tube is then centrifuged for 10 seconds and examined for agglutination both macroscopically and microscopically.

All serums that gave positive agglutination reactions in any of the three tubes were subjected to attempts at antibody identification.

Antibody Identification

The antibody identification method used in this study consists of testing the ability of patients' serums to agglutinate some but not all of the red blood cells supplied commercially by Spectra Biologicals, Inc., as the "Tencell Panel." Each serum that gave a positive agglutination reaction in the antibody detection test was tested against a "Tencell Panel" under the same conditions used in the antibody detection test: 1) in a saline medium at room temperature, 2) in a saline medium at 4° C., 3) in a saline medium at 37° C. to which proteolytic enzyme is added, 4) in an albumin-enriched saline medium after 30 minutes' incubation at 37° C., and 5) by the indirect antiglobulin method.

The antibody identification test is conducted with a set of saline tubes, a set of enzyme tubes and a set of albumin-antiglobulin tubes; there are 12 tubes (10x75 mm) in each set:

Saline set. One drop of the serum to be tested is put into each of the 12 tubes. To tube #1 is added one drop of cell #1 of the "Tencell Panel," to tube #2 is added one drop of cell #2 of the "Tencell Panel," and so forth through tube #10. To tube #11 is added one drop of the patient's cells, washed and suspended in saline. To tube #12 is added a drop of the service's Reagent Red Blood Cells. The set is allowed to stand for 20 minutes at room temperature. It is then centrifuged for 10 seconds at 3,400 rpm and examined macroscopically for agglutination. The cells are then resuspended; the set is refrigerated at 4° C. for 20 minutes and is again centrifuged for 10 seconds and examined macroscopically.

Enzyme set, method #1. This method was used with all samples acquired while the patients were hospitalized. One drop of the serum to be tested is put into each of the 12 tubes. Red cells are added to each of the tubes as described above. One drop of Low's papain is then added to each tube. The set is placed in a 37° C. water bath for 30 minutes, then centrifuged for 10 seconds and examined macroscopically.

Enzyme set, method #2. This method was instituted in 1965 and was used on all one-year follow-up serum samples. One drop of the serum to be tested is put into each of the 12 tubes. Red cells are added to each of the tubes as described above. One drop of Spectrazyme is then added to each tube. The set is

allowed to stand at room temperature for 3 minutes. It is then centrifuged and examined macroscopically. If there is no agglutination in the tube the cells are completely resuspended and the set is placed in a 37° C. water bath for 10 minutes. It is then again centrifuged and examined macroscopically.

Albumin-antiglobulin set. One drop of the serum to be tested is placed in each of the 12 tubes of the set. Red cells are added to each of the tubes as described above. One drop of a 22% solution of bovine albumin is then added to each tube and the contents of each tube is mixed thoroughly. The set is then centrifuged and examined macroscopically. The cells are then resuspended and the set is placed in a 37° C. water bath for 30 minutes, then again centrifuged and examined macroscopically. The cells are again resuspended and are washed three times with normal saline. A drop of antiglobulin serum (University of Pennsylvania) is added to each tube and the suspensions are thoroughly mixed. The set is then centrifuged for 10 seconds and examined for agglutination both macroscopically and microscopically.

After all reactions have been run, antibody identification is made, if possible, by comparison of the agglutination results with the known antigenic determinants on the various red cells of the "Tencell Panel."

Follow-up Procedure

As long as each patient stayed in the hospital a blood sample was taken from him every five days. Practical considera-

tions forced some deviations from this five-day interval between posttransfusion serum samples, but on the whole it was maintained as a standard. The numbers of patients followed and the length of time for which they were followed are shown in Table 10.

By the time of the one-year follow-up 39 (35%) of the 111 patients in the study had died. It was possible to secure one-year follow-up samples from 60 patients, representing 83% of those who were still living.

Results

Before transfusion eight of the 111 patients in the study were found to have antibodies. Four were not identifiable by our methods, two were anti-K (Kell), and one was anti-Rh_o(D), and one was anti-P. After transfusion, two patients developed antibodies while still hospitalized. One antibody was identified as an anti-M and the other as anti-c. A third patient was found to have an anti-K at the time of the one-year follow-up.

Discussion

Patient J.M. This 43-year-old white married man with a recent history of myocardial infaction and cerebral vascular accident suffered a pulmonary embolus and underwent emergency embolectomy at this hospital. His preoperative antibody detection test was negative. He had had no blood transfusions before operation. At operation he received 5 units of blood and he received three more during the first three days after operation. Six days after operation an antibody detection test

FOLLOW-UP of PATIENTS

Number of Patients	Duration of Posttransfusion Follow-up
20	35 days
6	30 "
10	25 "
11	20 "
10	15 "
32	10 "
22	5 "

TABLE 10

was negative. Ten days after operation an antibody detection test was positive and his antibody was identified as anti-M. The antibody titer was 128 using 22% bovine albumin as diluent and following the method described for the albumin-antiglobulin tube in the antibody detection test. A year after operation the antibody detection test was again negative.

Patient D.H. This 45-year-old married woman entered this hospital for the third time because of acute intestinal obstruction. On her first admission, in 1947, she underwent excision of an intervertebral disk with spinal fusion. Her chart shows that she received one unit of blood at operation. She had no other history of transfusion. At the time of her third admission an antibody detection test was weakly positive by the indirect antiglobulin method and in the enzyme tube. Testing against a "Tencell Panel" gave nonsensical results. The patient underwent decompressive colostomy and was discharged. A few weeks later, she was admitted to this hospital for the fourth time for laparotomy. She was found at operation to have carcinoma of the sigmoid colon and left colectomy was performed. She received three units of blood. Five days after operation an antibody detection test was weakly positive by the indirect antiglobulin method but negative in the enzyme tube. On the eighth postoperative day the antibody detection test was very strongly positive by the indirect antiglobulin method and in the enzyme tube. The antibody was identified as anti-c with an antiglobulin titer of 256 using 22% bovine albumin as diluent and following the albumin-antiglobulin tube method. This patient was group AB, $Rh_0(D)$-positive. Because of the rarity of compatible blood she was phlebotomized of one unit

before her discharge. This unit was stored and was returned to the patient's circulation to replace blood lost at closure of her colostomy three weeks later. A year after her colectomy the antibody detection test was weakly positive by the antiglobulin method and negative in the saline and enzyme tubes. Tested against a "Tencell Panel," her serum was found still to contain detectable levels of anti-c. Because 1) this patient received a transfusion in 1947 at the time of her first admission, and 2) her antibody detection test was strongly positive 8 days after transfusion in 1964, this may have been a secondary response.

Patient G. D. This 44-year-old white married man with no history of previous transfusion underwent elective closure of a ventricular septal defect. He received 16 units of blood at and after operation, including those used to prime the disc oxygenator. The preoperative antibody detection test was negative. Five days after operation he developed nonspecific cold agglutinins, but otherwise the antibody detection test was normal up to and including the time of discharge 15 days after operation. A year after operation this man was found to have a weak anti-Kell. The titer was less than one using normal saline as diluent and adding one drop of 22% bovine albumin to each tube. On the day before his discharge this patient had received two more units of blood, bringing his total exposure during this hospitalization to 18 units. It was possible to find and to type the donors of the two predischarge units -- one was Kell-positive and one Kell-negative. It seems reasonable that the patient was sensitized by the Kell-positive

predischarge unit, since the day before his discharge an antibody detection test had been negative, and he had received no transfusions for two weeks before that.

In calculating the rate of antibody occurrence those patients whose serums were tested only twice -- before transfusion and five days afterward -- were excluded because it is extremely unlikely that antibody would reach serologically detectable levels within five days after the first antigenic stimulus. The remaining 89 patients received a total of 332 units of blood, for an average of 3.7 units per patient. This is exactly equal to the average number of units given to each transfused patient over the five-year period reported on in Part I of this paper. Three of these patients (about 3%) developed antibodies after transfusion.

Three antibodies were produced in response to the total stimulus of 332 units. This is a rate of 0.009 antibodies per unit. Thus in this series of patients the chance of developing an irregular blood group antibody from a single transfusion was about 0.9%.

Lostumbo et al (25) studied a group of 127 patients who underwent open heart surgery. These patients were exposed to a total antigenic stimulus of 3160 units of blood. Thirty specific antibodies were formed in response to this total antigenic stimulus (cold panagglutinins and nonspecific antibodies are omitted from this comparison because they were omitted from all Yale-New Haven data), for a rate of 0.0095 antibodies per antigenic unit. In this group the chance of

developing an irregular blood group antibody from a single transfusion was about 0.95%.

Conclusions

Unfortunately it seemed necessary to eliminate from the study those 22 patients whose serums were tested for antibody only twice -- before transfusion and five days afterward. Nonetheless, the correlations between Part I and Part II of this study and between Part II and the work of others (4,25) tend to support the findings.

About 3% of the patients studied in Part II made new antibodies after transfusion. This figure agrees fairly well with the finding in Part I during the period when the service had reached a fairly high level efficiency; that is, of patients who were transfused and who were expected to be transfused, 4.78% in 1964 and 3.73% in 1965 had isoimmune antibodies in their serums (Table 6).

The 0.9% risk of developing an antibody as a result of a single transfusion is this series correlates very well with Giblett's calculated risk of 1% (4), and with the findings of Lostumbo et al (25). The suggestion here is that as transfused patients in this hospital receive an average of almost 4 units each, about 4% of them can be expected to develop antibodies.

An incidental finding of great interest was that if one follows a random group of transfused patients for a year, at the end of that time 35% of them will be dead.

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